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Aromatic profiles of *Thymus hyemalis* and Spanish *T. vulgaris* essential oils by GC–MS/GC–O

K.L. Goodner [a,*], K. Mahattanatawee [a], A. Plotto [a], J.A. Sotomayor [b], M.J. Jordán [b]

[a] *USDA, Citrus and Subtropical Products Laboratory, 600 Avenue S NW, Winter Haven, FL 33881, USA*
[b] *IMIDA, Departamento de Recursos Naturales y Desarrollo Rural. C./ Mayor s/n, 30150 La Alberca Murcia, Spain*

Abstract

Thymus hyemalis L. and Spanish *T. vulgaris* subsp. *vulgaris* shrubs are characterized by a great chemical intraspecific variability among plants. This fact makes it difficult to detect real changes occurring in their essential oil composition during the vegetative cycle. Because of this, clones of *T. hyemalis* and *T. vulgaris* were used to monitor seasonal variations in their aromatic composition of the essential oil. Shrubs were harvested at five different phenological stages during the plant vegetative cycle. Capillary GC/MS with olfactometry analysis was used to determine the volatile profile of the essential oil samples. GC–olfactometry analysis was conducted on each phenological stage for *T. hyemalis* and *T. vulgaris*. A total of 96 (*T. hyemalis*) and 52 (*T. vulgaris*) aromas were detected with the 27 (*T. hyemalis*) and 26 (*T. vulgaris*) most important reported here. The compounds with the most aroma impact for *T. hyemalis* were linalool, borneol, thymol, and β-damascenone. The compounds with the most aroma impact for *T. vulgaris* were eucalyptol, borneol, terpinyl acetate, and β-damascenone. β-ionone, myrtenol, and β-damascenone are reported for the first time.

Keywords: *Thymus hyemalis*; *Thymus vulgaris*; Thyme; Phenological stages; Olfactometry

1. Introduction

Thyme is a 10–30-cm tall aromatic plant native to the Mediterranean region. It is commonly sold fresh, ground, dried, or as an essential oil (Anonymous, 1996). Spain is a large supplier of thyme, exporting approximately 1.6 million kg of thyme, and between 2 and 3 million kg of thyme essential oil (Blanco et al., 1998). Approximately one-third of the thyme imported into the United States is from Spain (USDA, 1998).

This work focuses on two species of the *Thymus* genus: *Thymus hyemalis* and *T. vulgaris* subsp. *vulgaris*. *T. hyemalis* Lange, or winter thyme is grown mainly in Alicante, Murcia, and Almeria from sea level to approximately 400–700 m above sea level. Volatile research on this species has been done (Cabo et al., 1986, 1987; Sáez, 1995, 1998; Jordán et al., 2003). Thymol, carvacrol, borneol, and linalool were the most abundant compounds reported.

T. vulgaris subsp. *vulgaris* (chemotype 1,8-cineole) is grown from sea level to 2000 m above sea level (Morales, 1986). Over 60 compounds have been identified in *T. vulgaris*, including thymol (72,900–482,600 ppm), carvacrol (10,000–63,800 ppm), 1,8-cineole (2500 ppm–26,200 ppm) (Nijssen et al., 1999). Experiments with this species have been performed worldwide, including Mongolia (Shatar and Altantsetseg, 2000), Cuba (Pino et al., 1997), Kenya (Maitai and Talalaj, 1981), New Zealand (McGimpsey

* Corresponding author. Tel.: +1 863 293 4133x127; fax: +1 863 299 8678.
E-mail address: goodner@citrus.usda.ogv (K.L. Goodner).

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et al., 1994), Italy (Piccaglia and Marotti, 1991), Canada (Letchamo et al., 1999), and Spain (Guillen and Manzanos, 1998).

T. hyemalis and *T. vulgaris* clones were cultivated to determine seasonal variations in the aroma profile of the essential oils using gas chromatography/olfactometry (GC–O), in order to provide information on optimum harvesting period to commercial growers, based on industrial requirements.

2. Materials and methods

2.1. Plant material

Thyme species were propagated by top cuttings of plants already cultivated in the experimental area of the IMIDA (Murcian Institute of Investigation and Agricultural Development) at Torreblanca (37°47′N–0°54′W and 30 m above sea level) in the region of Murcia, Spain. Stems were planted in alveolar trays and they were kept in greenhouse conditions until the root system was substantially developed. All the plants were transplanted onto the experimental area in the autumn of 2001. Harvesting of five shrubs per species was made in each of the five phenological stages during the entire vegetative cycle: vegetation, full bloom, full bloom/at the beginning of fruit maturation, fruit maturation, and advanced fruit maturation stages.

2.2. Essential oil extraction

Five plants from each thyme species were harvested at each phenological stage, making a total of 25 plants per species. The aerial parts (above-ground) of dry individual plants were steam distilled for 3 h using a Clevenger-type system. Samples were dried with anhydrous sodium sulfate and kept in amber vials at 4 °C until chromatographic analysis.

2.3. Gas chromatography–mass spectrometry-olfactometry

Essential oil samples of 0.1 μl were injected neat (directly) into an Agilent 6890N gas chromatograph (Palo Alto, CA, USA). The split ratio was 10:1 onto a 30 m × 0.25 mm HP-5 (cross-linked phenyl–methyl siloxane) column with 0.25 μm film thickness (Agilent, Palo Alto, CA, USA) to a mass spectrometer and sniff port. The column, with a flow rate of 1.9 ml/min, was initially at 50 °C, increased to 180 °C at a rate of 2.5 °C/min, then increased to 250 °C at a rate of 100 °C/min and held for 7.3 min for a total run time of 60 min. The injector was held at 250 °C and the MS transfer line was held at 280 °C. The effluent was split 3:1 between a Gerstel Olfactometry Detection Port (ODP) (Baltimore, MD, USA) and an Agilent 5973N mass spectrometer (Palo Alto, CA, USA). The mass spectrometer scanned from 45 to 250 *m/z* with an ionization energy of 70 eV. Samples were detected at the sniff port (GC–O) in triplicate runs with descriptors noted along with an estimated intensity (0–10) and retention time. Intensities were summed over the three runs to get an overall intensity for the peaks in each sample (0–30). A retention index was generated by using a series of *n*-alkanes from C-5 to C-19.

3. Results and discussion

Determining the best harvest period for thyme plants to obtain the desired essential oil quality is difficult due to the many variables that can affect the product such as growing conditions such as weather, irrigation, fertilizers along with harvest and post-harvest handling and processing conditions. This study used clones of *T. hyemalis* and *T. vulgaris* plants to isolate changes that occurred during the vegetative cycle.

3.1. T. hyemalis essential oil

The results of the GC–olfactometry of *T. hyemalis* are presented in Table 1. A total of 96 aromas were detected in the 15 analyses (5 vegetative states, 3 replicates per extract), but only 27 are described in the table. Those included in the table were the most consistent aromas detected. Many detected aromas were only smelled in a single run of a single vegetative state. This could likely mean that they were false positive detections and were, therefore, not included. Further study needs to be conducted to determine whether some of these possible aromas are factual. Based on the retention index, mass spectrum, and aroma descriptor, tentative identifications have been made.

There are not very many trends that are immediately apparent. This has to do with the precision, which varies between panellists, of GC–O which due to the human assessor is not as precise as instrumentation. Additional panellists will analyze these samples in future studies. In this preliminary analysis, five aromas show a trend. An aroma at retention index 972 with a descriptor of "engine oil, green-burning" is identified as β-myrcene. This aroma showed an upward trend that doubled in intensity as the plant matured. The next two compounds at retention indices 1061 and 1133 had an "herbal, menthol" and "spices, citrusy" aromas, respectively. Both compounds, along with others, are listed as unknown because

Table 1
GC–O intensities for *T. hyemalis* essential oil, with retention index, descriptor, and chemical identification for the five maturation stages

RT	RI	Descriptor	Chemical	Veg	FB	FB-FR	FR	AFR
3.70	776	Fruity	Methyl 2-methyl butyrate	15	13	13	16	13
5.76	846	Cooked potato, grain	Unknown	19	15	15	20	18
9.92	972	Engine oil, green-burning	β-Myrcene	8	13	20	24	18
11.71	1021	Engine oil, green-menthol, menthol	p-Cymene	20	18	20	28	26
12.00	1028	Menthol	Eucalyptol	5	12	4	7	4
12.74	1048	Engine oil	β-Ocimene	2	0	2	0	2
13.15	1058	Green-menthol	γ-Terpinene	8	13	7	0	11
13.29	1061	Herbal, menthol	Unknown	10	5	3	4	0
15.11	1106	Floral	Linalool	28	30	27	30	30
16.28	1133	Spices, citrusy	Unknown	10	11	5	6	2
16.53	1139	Green, menthol, citrusy	Unknown	16	14	9	22	10
17.91	1169	Herbal, menthol	Unknown	9	8	11	12	8
18.34	1179	Earthy, herbal, menthol	Borneol	30	25	30	30	28
19.02	1193	Plastic	Terpine-4-ol	3	4	0	10	6
22.37	1262	Menthol, thyme, green	Unknown	14	17	13	21	20
22.66	1267	Thyme, green musty, menthol, spice	Unknown	17	16	11	22	20
24.96	1312	Herbal, menthol, green	Bornyl acetate	15	16	23	28	25
25.29	1318	Thyme, green, menthol	Thymol/carvacrol	30	30	30	30	30
28.43	1377	Floral, berry, spices, clove	Eugenol	6	9	11	19	16
29.41	1395	Sweet, honey	β-Damascenone	28	30	28	30	30
30.83	1421	Sweet	β-Caryophyllene	6	16	12	13	18
34.21	1485	Raspberry	β-Ionone	11	17	13	12	20
37.87	1559	Spice	Unknown	3	5	6	9	2
38.23	1566	Minty, fatty	Spathulenol	7	2	0	0	5
38.79	1578	Fatty	Unknown	2	3	0	5	2
39.97	1603	Menthol	Unknown	4	8	3	7	4
40.81	1622	Menthol	Unknown	3	2	0	6	5
41.42	1635	Fatty	Unknown	2	2	6	3	6

RT: Ret. time; RI: Ret. index; Veg: vegetative; FB: full bloom; FB-FR: fruit bloom/maturation; FR: fruit maturation; AFR: advanced fruit maturation.

agreement among the retention index, mass spectrum, and aroma descriptors was not similar enough to ensure accuracy. Both compounds had drastic decreases (100% and 80%, respectively) in aroma intensity as *T. hyemalis* matured. Two later eluting compounds at 1377 and 1421 retention indices displayed increasing aroma intensity, about two to three times the intensity in the vegetative cycle. These compounds were identified as eugenol and β-caryophyllene. Additionally, two unknowns (RI 1262 and 1267) and bornyl acetate were largest during the advanced fruit maturation stage with increases of about 50–80% from the lowest observed intensities.

Two compounds of note are thymol and carvacrol. There was not sufficient resolution in the GC–O to differentiate these two compounds because they have similar odor. The compounds were separated chromatographically, but are sufficiently close so that it was difficult for a human assessor to differentiate between the two compounds.

Two compounds, β-damascenone and β-ionone, were only identified by retention index and aroma descriptor. The compounds were at very low concentrations and could not be identified by the mass spectrometer, but were still observed by the human assessor due to the very low aroma thresholds (low ppb) of β-damascenone and β-ionone. Even though they were not detected by the mass spectrometer, the aroma intensities were high.

The most intense aromas observed were those from linalool, borneol, thymol/carvacrol, and β-damascenone. Each of these compounds resulted in an observed intensity of near the maximum of 30. Only thymol/carvacrol had an intensity of 30 in each maturation stage. It is likely that the thymol is significantly above the maximum intensity. There were several compounds that were of intermediate intensity: β-myrcene, *p*-cymene, bornyl acetate, and three unknowns at retention indices 846, 1262, and 1267.

Thymol/carvacrol and p-cymene were the two compounds that were only observed in *T. hyemalis* and not *T. vulgaris*. Both compounds have high intensity with thymol/carvacrol having the maximum intensity throughout the maturation stages and *p*-cymene being a moderately intense aroma and increasing during the last two stages of development.

Table 2
GC-O intensities for *T. vulgaris* essential oil, with retention index, descriptor, and chemical identification for the five maturation stages

RT	RI	Descriptor	Chemical	Veg	FB	FB-FR	FR	AFR
3.72	777	Fruity	Methyl-2-methyl butyrate	6	2	2	0	2
6.26	862	Green	Hexanol	0	0	0	0	2
10.06	976	Sweet, green, menthol	β-Myrcene	15	17	19	12	23
11.67	1020	Sweet, menthol	Eucalyptol	30	30	30	30	30
13.15	1058	Green, menthol	γ-Terpinene	7	2	3	0	0
15.11	1106	Floral	Linalool	30	28	30	30	30
17.35	1157	Green, menthol, sweet	Camphor	12	0	0	2	2
18.38	1179	Herbal, menthol, woody	Borneol	30	30	30	30	30
19.33	1200	Solventy	Cryptone	6	3	11	8	10
19.49	1203	Engine oil	α-Terpineol	2	0	0	3	14
19.72	1208	Mint	Myrtenol	6	4	2	0	0
22.47	1263	Mint	Linalyl acetate	7	0	0	6	2
23.07	1275	Sweet spices	Bornyl acetate	13	16	17	16	18
25.92	1330	Menthol, green, citrusy	Unknown	18	0	2	7	10
27.71	1363	Spice/pepper, herbal, citrusy	Terpinyl acetate	30	30	30	30	30
28.35	1375	Spice, clove	Eugenol	24	26	26	18	2
29.39	1395	Sweet, honey	β-Damascenone	30	30	30	30	30
30.86	1422	Sweet, floral	β-Caryophyllene	20	17	19	17	17
34.25	1486	Raspberry	β-Ionone	16	4	16	15	16
37.83	1558	Metallic, green, fatty	Unknown	10	5	10	8	5
38.42	1570	Herbal, menthol	Spathulenol	8	0	0	0	2
38.51	1572	Spices, musty	Caryophyllene oxide	0	0	0	2	2
38.68	1575	Sweet	Unknown	6	0	0	4	2
39.94	1602	Menthol, fatty, engine oil	Unknown	6	2	0	4	0
40.24	1609	Spices, clove	Unknown	10	0	8	4	2
41.45	1636	Metallic	Unknown	4	10	13	5	2

RT: Ret. time; RI: Ret. index; Veg: vegetative; FB: full bloom; FB-FR: fruit bloom/maturation; FR: fruit maturation; AFR: advanced fruit maturation.

3.2. Spanish *T. vulgaris* essential oil

The results of the GC–olfactometry of *T. vulgaris* are presented in Table 2. A total of 52 aromas were detected in the 15 analyses (5 vegetative states, 3 replicates each), but only 26 are described in the table. Those included in the table were the most consistent aromas detected. As for *T. hyemalis*, many detected aromas were only smelled in a single run of a single vegetative state, possibly false positive detections and were, therefore, not included. Based on the retention index, mass spectrum, and aroma descriptor, tentative identifications have been made.

T. vulgaris was similar to *T. hyemalis* with few readily apparent trends in the GC–O data. A "green, menthol" aroma at retention index 1058, identified as γ-terpinene, decreased with maturity. Caryophyllene oxide, a minor odorant, with a retention index of 1572 and aroma descriptor "spices, musty" was not detected until the last two stages of maturity. The biggest change can be seen in eugenol (RI = 1375) that dropped in intensity dramatically with fruit maturation stage and was almost non-existent in the advanced fruit maturation stage.

As with *T. hyemalis*, β-damascenone and β-ionone are important aroma impact compounds that were not detected by the mass spectrometer. These compounds will have to be quantitated using other sampling methods to increase their concentrations to a level that can be detected by the instrument. Neither compound has been reported in these *Thymus* species before. Another compound reported here for the first time in *T. vulgaris* subsp. *vulgaris s* is myrtenol. This compound has a retention index of 1208 and an aroma of "mint". The retention time, mass spectrum, and aroma descriptor matched those published in the literature.

There are several compounds that are either only in *T. vulgaris*, or at much higher aroma intensities values than *T. hyemalis*. These compounds are eucalyptol, terpinyl acetate, eugenol, and β-caryophyllene. It is apparent that these constitute the major differences in aroma between these two essential oils.

The most intense aromas observed were those from eucalyptol, linalool, borneol, terpinyl acetate, and β-damascenone. Each of these compounds resulted in an observed intensity of near the maximum of 30. Eucalyptol, terpinyl aceate, and β-damascenone had intensities of 30 in each maturation stage. It is likely that these

compounds are above the maximum intensity. There are several compounds that are of intermediate intensity: β-myrcene, bornyl acetate, and β-caryophyllene.

These results highlight the fact that the aroma profiles of the two thyme essential oils vary depending on the vegetative stage at harvest which would enable growers/processors to custom tailor the thyme essential oil to their customer's specifications.

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